Reference: *Biol. Bull.*, **151**: 306–313. (October, 1976)

SEASONAL VARIATIONS OF SODIUM AND POTASSIUM CONCENTRATIONS IN DIFFERENT PARTS OF THE FROG MYOCARDIUM

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Changes in environmental conditions result in alteration of the physiological parameters in amphibians to a greater extent than in higher animals. Changes in the body temperature of the frog, in different seasons, are associated with variations in the volume and electrolyte content of extracellular and intracellular fluids (Deyrup, 1964), with alteration of the function of the frog kidneys (Höber, 1927; Oliver and Shevky, 1929), and with altered responsiveness of the frog skin and kidneys to pituitrin (Hong, 1957). Schmidt-Nielsen and Forster (1954) found higher water content and increased extracellular volume in the frog under cold conditions than at room temperature. Maurer (1938) also observed increased extracellular volume in winter. Others (Churchill, Nakazawa and Drinker, 1927) found similar changes of extracellular volume in the male frog during the breeding period.

Questions arise whether these fluctuations are associated with variations in the ion content of the different tissues as well, and what is the relationship between the electrolyte concentration of these tissues and the season in which the experiment is done. Answers can be provided by statistical testing of groups of experimental data obtained in different seasons of the year regarding the two alternatives: whether the difference between any two values for ion concentrations of the tissues is due to the seasonal variation or to influence of other experimental circumstances.

The purpose of the present investigation is to determine the sodium and the potassium concentrations in various parts of the frog myocardium (sinus venosus, atrium and ventricle) in the course of a year.

Materials and Methods

The investigations of this study cover a two-year period, but, for technical reasons, no data are available for the two summer months, July and August. Female frogs of the species *Rana esculenta* were used for the experiments. The animals, weighing 30–60 g, were kept after removal from their natural environment in an open vessel with running tap water for 3–10 days prior to the experiment. Three to four hours before the experiment, the animals were transferred to a glass container with shallow water at room temperature (19–23° C).

The animals were decapitated and the spinal cord destroyed. The heart was perfused for 40–60 minutes with Ringer-solution *via* the inferior vena cava and the aorta at room temperature. The composition of the perfusate was as follows: Na^+, 113.5 mEq/l; K^+, 1.8 mEq/l; Ca^{2+}, 2.0 mEq/l; Cl^-, 114.8 mEq/l; HCO_3^-, 2.4 mEq/l; and glucose, 5.5 mmole/l. After the termination of the perfusion, hearts were separated into sinus venosus, atrium and ventricle. Each part was weighed and dissolved in concentrated nitric acid. Samples were brought to a known volume, and sodium and potassium concentrations were determined by flame photometry.

The ion concentrations found in each part of the heart were expressed as μEq/g wet weight and were plotted against the date on which the experiments had been performed. For two periods of the year, from January 1 to June 30 and from September 1 to December 31, a linear relation was calculated between the date of the experiment and the concentrations found in the different parts of the heart. Each month was considered to have 30 days, and the date of the experiment expressed as the number of elapsed days from the beginning of the series.

Since the sodium concentration is expressed as a function of date, the concentration values for each part were approximated using the formula, $f(t) = k \sin at + c$, where a is constant defining the period; k and c were then arrived at by applying the principle of the least squares.

Taking the period as one year, having 360 days, $a = \pi/180$; thus the formula takes the form $f(t) = k \sin (\pi/180)\, t + c$.

Since the minimum of the sodium concentration falls on the winter period, April 1 is to be taken as the first day of the period of the function ($t = 1$).

If on t_i day c_i concentration value is measured, then to determine k and c one has to minimize the sum of the squares: $Q(k, c) = \Sigma_i\, (k \sin \pi/180\, t_i + c - c_i).^2$

At any rate $Q(k, c)$ does have a minimum, which can be obtained by solving the equations by partial derivatives. $\partial Q/\partial k = 0$ and $\partial Q/\partial c = 0$. Thus we have $\partial Q/\partial k = 2\, \Sigma_i\, (k \sin \pi/180\, t_i + c - c_i) \sin \pi/180\, t_i = 0$ and $\partial Q/\partial c = 2\, \Sigma_i\, (k \sin \pi/180\, t_i + c - c_i) = 0$. Hence the solution:

$$k = \frac{n \sum_i c_i \sin \frac{\pi}{180} t_i - (\sum_i c_i)\left(\sum_i \sin \frac{\pi}{180} t_i\right)}{n \sum_i \left(\sin \frac{\pi}{180} t_i\right)^2 - \left(\sum_i \sin \frac{\pi}{180} t_i\right)^2},$$

$$c = \frac{\sum_i c_i - k \sum_i \sin \frac{\pi}{180} t_i}{n}.$$

Results

There is a definite sodium concentration gradient through the anatomical areas of the heart, and the potassium concentration gradient is reversed (Table I). The linear correlation coefficients between the ion concentrations and the periods of the observation are summarized in Table II. It is seen that in the first half of the year, the values for sodium concentrations are positive and highly significant in each part of the heart, whereas in the second half the values are negative and differ significantly from zero.

Potassium concentration fails to show similar variations. Except for the diminishing ventricular potassium concentration observed in the second half of the year, there is no significant correlation between potassium concentrations and the experimental periods. The "best squared" sine curve is also shown in Figures 1, 2, and 3. The value of c for the different parts of the heart is very near to appropriate averages summarized in Table I. This results from the fact that the indi-

TABLE I

Sodium and potassium concentrations in the different parts of the frog heart (μEq/g wet weight of tissues).

	Mean ± s.d. (number of experiments)	
	Na	K
Sinus venosus	106.2 ± 28.6 (57)	22.6 ± 6.1 (49)
Atrium	75.7 ± 17.4 (67)	29.2 ± 6.1 (49)
Ventricle	50.8 ± 11.3 (64)	55.7 ± 7.5 (49)

vidual experiments are dispersed nearly equally in both halves of the year, and therefore the value of $\Sigma_i \sin \pi/180\, t_i$ is very small.

The k parameter, which determines the amplitude of the function, constitutes 23.6% of the value of c in the case of the sinus venosus; it is only 15.2% for the atrium and 17.0% for the ventricle.

DISCUSSION

The sodium and potassium concentration gradients between different parts of the heart muscle observed in amphibians can be found in phylogenetically higher species (*i.e.*, in mammals), too. Holland (1960) found the sodium concentration to be 190–210 mEq/kg wet tissue in the sinoauricular node region of the rabbit heart, 130 mEq/kg in the right ventricle and 110 mEq/kg in the left ventricle, while the potassium concentration was 42 mEq/kg, 67 mEq/kg and 70 mEq/kg,

TABLE II

Correlation coefficients between sodium and potassium concentrations, and the time of the experiments.

		Na		K	
		First half of the year	Second half of the year	First half of the year	Second half of the year
Sinus venosus	r	0.54	−0.61	−0.31	−0.08
	n	36	21	31	18
	P	<0.001	<0.001	0.1 < *P* < 0.2	0.7 < *P* < 0.8
Atrium	r	0.46	−0.83	0.35	−0.38
	n	45	22	31	18
	P	<0.001	<0.001	0.05 < *P* < 0.1	0.1 < *P* < 0.2
Ventricle	r	0.60	−0.61	0.11	−0.61
	n	42	22	31	18
	P	<0.001	<0.001	0.5 < *P* < 0.6	0.001 < *P* < 0.01

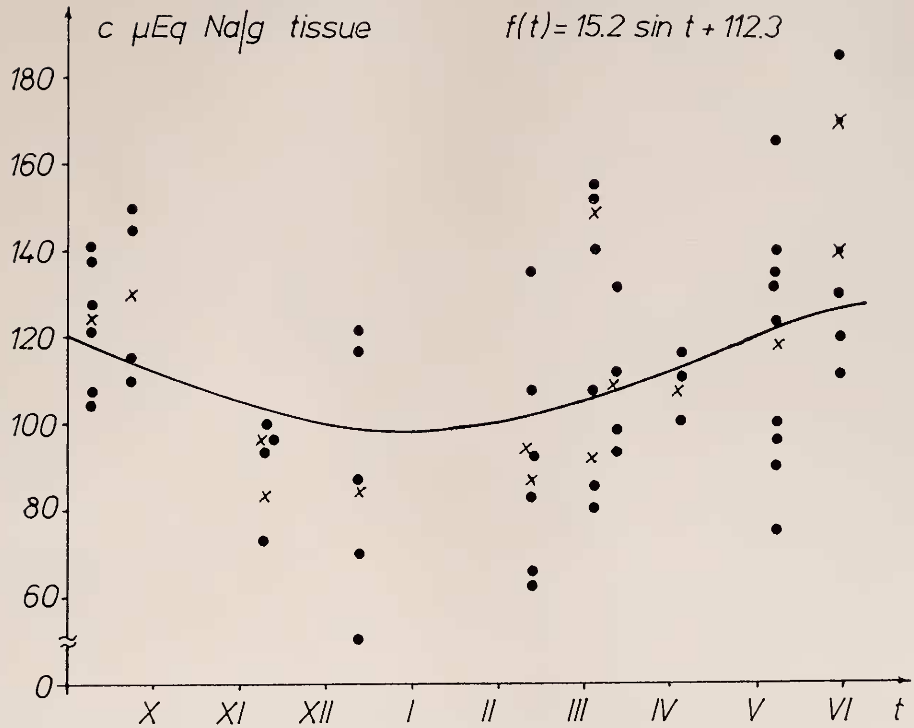

FIGURE 1. Sodium concentration of sinus venosus at different times of the year. Closed circles represent individual concentration values; crosses indicate mean values of the one-day data. A fitted sine curve is also shown.

respectively. The sodium concentration in the auricle of the human heart averaged 95.7 mEq/kg tissue, in the atrium 89.2 mEq/kg, and in the ventricle 51.3 mEq/kg. The potassium concentration, on the other hand, was 38.9 mEq/kg, 38.4 mEq/kg, and 64.8 mEq/kg, respectively (Jansen and Stappenbeck, 1962).

In previous studies (Harza, Erödi and Hársing, 1966; Harza, Váradi and Erödi, 1971) similar values for ion concentrations were reported. The values for inulin space (37.1%, 35.5% and 27.1%) and for water content (83.9%, 86.1% and 88.6%) were obtained from January 1 to April 15. By taking into account the ion concentration values obtained during this period of the year (106.0 μEq/g, 73.4 μEq/g, 45.0 μEq/g wet weight for sodium and 25.0 μEq/g, 28.0 μEq/g, 53.2 μEq/g wet weight for potassium) the intracellular ion concentrations can be estimated. The data (136, 63, 23 μEq/ml of intracellular water for sodium and 52, 54, 86 μEq/ml of intracellular water for potassium, respectively) suggest the need for another explanation of ion content values apart from the differences in the extracellular space. These findings are discussed in detail elsewhere (Harza, *et al.*, 1966; Harza *et al.*, 1971).

Correlation coefficients were calculated, after dividing the year arbitrarily into two equal periods. Sodium concentrations in all three parts of the heart increase continuously from the beginning of January to the end of June, while they decline

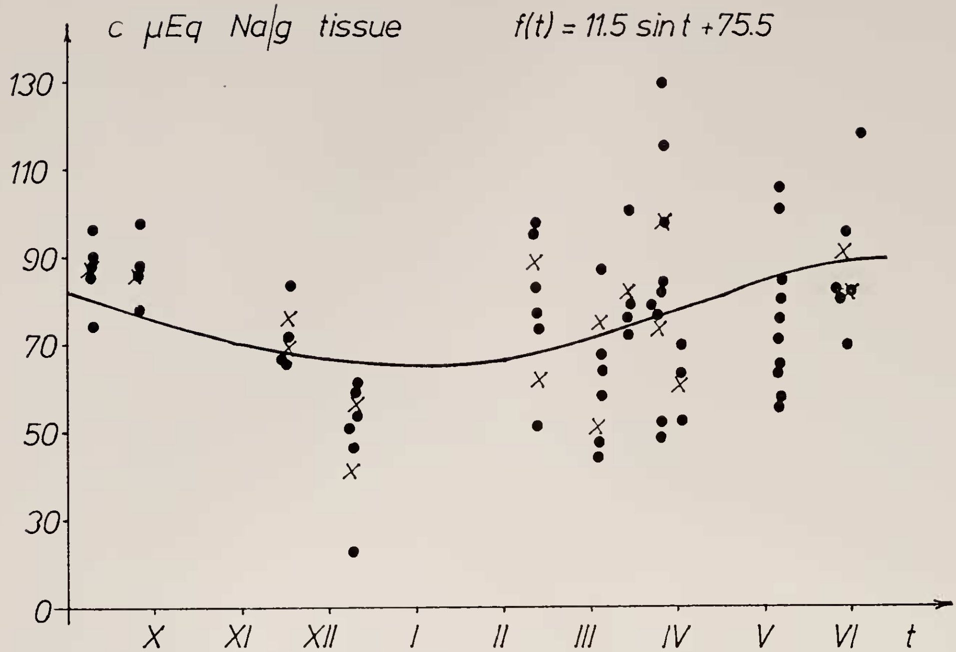

FIGURE 2. Sodium concentration of the atria at different times of the year. Closed circle represents individual concentration values; cross mark indicates mean value of the one-day data. A fitted sine curve is also shown.

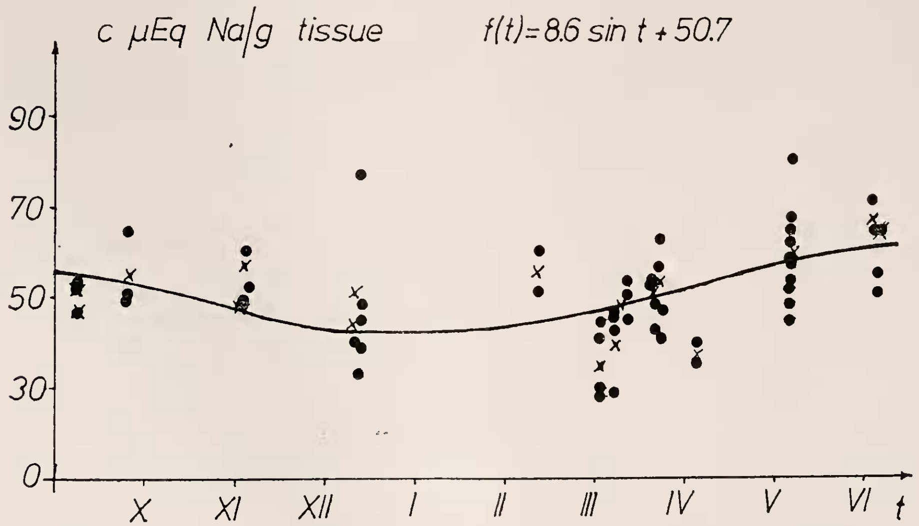

FIGURE 3. Sodium concentration of the ventricle at different times of the year. Closed circle represents individual concentration values; cross mark indicates mean value of the one-day data. A fitted sine curve is also shown.

from the beginning of September to the end of December. Therefore, the periods from January 1 to June 30 and from September 1 to December 31 were treated separately. Linear correlation coefficients can show not only the existence of correlation but can also indicate the direction of change as well. Therefore, linear correlation coefficients were calculated despite the fact that there was no evidence for an overall linearity in the "time *vs.* concentration" relationship. The deviation of the coefficients from zero in all three parts of the heart confirms the arbitrary division of the year set up by us.

No similar periodicity could be detected in potassium concentration. Therefore, the average values and standard deviation for the potassium concentration *obtained in any part* of the year can be used, given our experimental conditions.

However, the statistical treatment of the averages and standard deviations for sodium concentration does need special care for two reasons. First, standard deviation could be influenced (widened) by seasonal changes in the case of an experimental series extending over a longer period. Secondly, statistical comparison between averages, of two groups obtained at different seasons of the year, should not be carried out by applying *t* test. The following procedure is suggested. From the individual data for the concentrations obtained in different parts of the year, in both experimental groups, values of c can be calculated using the following equation:

$$c = \frac{\sum_i c_i - k \sum_i \frac{\pi}{180} t_i}{n}.$$

Since the value of c does not depend on the time of the experiment, a significant difference between values of c, calculated by some appropriate mathematical statistical method (*e.g.*, by applying *z* statistic; Dixon and Massey, 1969) from the experimental and control readings, could demonstrate the effect of the experimental procedure. (The calculation of the standard deviation is based on the equation:

$$s = \left[\frac{\sum_i [f(t_i) - c_i]^2}{n - 1}\right]^{\frac{1}{2}}.\Bigg)$$

Experimental conditions (for example, removal of the animals from their natural environment prior to the experiment, not taking special care of their feeding, and the constancy in the composition of the perfusion fluid irrespective of the seasons) might raise some doubts regarding the *biological* significance of our data. However, if experimental conditions had any effect on the ion content of tissues, they did not cancel out the natural seasonal oscillations of sodium concentration. However, the lack of periodical fluctuations of potassium concentration could result from standard experimental conditions.

It is worth noting that in our earlier work we could not demonstate any changes in the inulin space and water content during a four-month experimental period.

The differential changes in sodium and potassium concentrations exclude, too, the possibility that changes in the water content are *solely* responsible for changes in cation concentration. For this and other reasons, possible fluctuations of the inulin space can not be an important factor in seasonal variation of ion content. In the sinus venosus the intracellular sodium concentration is a little higher than the extracellular one, while in the ventricle the cell water contains less sodium than the extracellular one. Therefore, sodium concentrations would have to change in opposite directions in the sinus venosus and ventricle with change in extracellular space.

However, data reflecting the seasonal fluctuations of sodium metabolism in the frog would suggest a more likely explanation. Knapowsky (personal communication, Dept. of Pathophysiology, Med. Acad., Poznan, 1973) has shown that the increased temperature results in an appreciable increase of sodium transport in the frog skin in late spring and early summer, whereas temperature has no effect on the transepithelial movement of sodium in autumn.

At lower temperatures the frog increases its body weight (Schmidt-Nielsen and Forster, 1954; Jørgensen, 1950a, 1950b). This results directly from water gain, occurring in two steps. The first, following the decrease of the temperature, is a rapid absorption, which is manifested even if the frog is kept in distilled water indicating a process independent from the ion movement. Therefore the plasma electrolyte concentration of the animals falls from 111 mM/l at room temperature to 90 mM/l at cooler temperatures (Schmidt-Nielsen and Forster, 1954). This initial, rapid water intake is followed by a slower one which is acompanied by inward movement of sodium. No net water intake could be demonstrated at higher temperatures. Disturbance of the frogs and removal from their aqueous environment results in the rapid loss of sodium and water. Excretion of the electrolyte occurs *via* the kidneys (Jørgensen, 1950a). Under these circumstances, sodium concentration in the plasma and the tissues must be lower at a lower temperature than at a higher one. Since there is no significant net transport of potassium either in or out. the result of water intake in the cold and of water loss under experimental conditions is reflected in the relative stability of the potassium concentration in the plasma and tissues.

Summary

Sodium and potassium concentrations in different parts of the frog heart (sinus venosus, atrium and ventricle) were investigated at various seasons of the year. The sodium concentration showed a marked periodicity in all three parts of the heart: minimum in winter, maximum in summer. Potassium concentration showed no seasonal variations, except for reduction in potassium concentration of the ventricle in the second half of the year.

The experimental results for sodium can be fitted by a sine curve, the differences between minimum (in winter) and maximum (in summer) representing 46.4% (for sinus venosus), 30.4% (atrium) and 34.0% (ventricle) of the spring-autumn values.

Variation in the electrolyte concentration could be explained by changes in salt and water metabolism induced by fluctuations in the temperature on one hand, and by the removal of the animals from their natural environment on the other.

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